US-EPA (1984): Health Assessment Document for Manganese. Final Report. Environmental Criteria and Assessment Office. Cincinnati OH.

Wasserman M, Mihail G (1961): Recherches concernant la pathologie professionnelle des mineurs des mines de manganèse. Arch Gewerbepath Gewerbehyg 18:632–657.

Whitlock CM, Amoso SJ, Bittenbender JB (1966): Chronic neurological disease in two manganse steel workers. Am Ind Hyg Assoc J 27:454–459.

WHO (1981): Environmental Health Criteria 17: Manganese. Geneva.

Yuwiler A, Plotkin S, Geller E, Ritvo ER (1970): A rapid accurate procedure for the determination of serotonin in whole human blood. Biochem Med 3:426–431.

tzias GC, Greenough JJ. The high specificity of the manganese pathway through the body. *J Clin Invest* 1958; 37:1298
tzias GC, Miller ST, Nicholson Jr AR, Maston WH, Tang LC. Prolongation of the life-span in mice adapted to large amounts of L-dopa. *Proc Nat Acad Sci USA* 1974; 71:2466-2469
tzias GC, Miller ST, Papavasiliou PS, Tang LC. Interaction between manganese and brain dopamine. *Med Clinics North Amer* 1976; 60(4):729-738
tzias GC, Miller ST, Tang LC, Papavasiliou PS, Wang YY. Levodopa, fertility and longevity. *Science* 1977; 196:349-351
tzias GC, Papavasiliou PS. Therapeutic studies of Parkinsonian patients long-term effect of DL and L-dopa. *Excerpta Med Found Inst, Congr Sec 175: Progr Neuro-Genet*, 1967; 1:357-365
tzias GC, Papavasiliou PS, Gellene R. Modification of Parkinsonism: Chronic treatment with L-dopa. *New Engl J Med* 1969; 280:337-345
tzias GC, Tang LC. An adenylate cyclase of brain reflects propensity for breast cancer. *Science* 1977; 197:1094-1096
tzias GC, Tang LC, Miller ST, *et al.* A mutation influencing the transportation of manganese, L-dopa, and L-tryptophan. *Science* 1972; 176: 410-412
tzias GC, Van Woert MH, Schiffer LM. Aromatic amino acids and modification of Parkinsonism. *New Engl J Med* 1967; 276:374-379
lay J, Diniker P. Drug induced extrapyramidal syndromes. In: *Handbook of Clinical Neurology*, Vinkers PJ, Bryun GW eds., 1968, Amsterdam & North Holland, 6:248-266
uffy PE, Tennyson VM. Phase and electron microscopic observations of Lewy Bodies and melanin granules in the substantia nigra and locus cerrules in Parkinson's disease. *Neuropathol Exp Neurol* 1961; 24:298
ringer H, Hornykiewicz O. Distribution of noradenaline and dopamine (3-hydroxytyramine) in the human brain and their behavior in diseases of the extrapyramidal system. *Klin Wochenschr* 1960; 38:1236-1239
Fehling C. Treatment of Parkinson's syndrom with L-dopa. A double blind study. *Acta Ne rol Scand* 1966; 42:367
Fore H, Morton RA. Manganese in rabbit tissu *Biochem J* 1952a; 51:600
Fore H, Morton RA. Manganese in eye tissu *Biochem J* 1952b; 51:603
Hurley LS, Everson GJ. Delayed development righting reflexes of offspring of manganes deficient rats. *Proc Soc Expil Biol Med 19 102:360*
Lai JCK, Leung TKC, Lim L. Differences in t neurotoxic effects of manganese during deve opment and aging: Some observations on bra regional neurotransmitter and non-neurotran mitter metabolism in a developmental rat mod of chronic manganese encephalopathy. *Neur toxicology* 1984; 5(1)
Mena I, Court J, Fuenzalida E, *et al.* Modificatio of chronic manganese poisoning. *New Eng Med* 1970; 282:5-10
Neff NH, Barrell RE, Costa E. Selective depletio of caudate nucleus dopamine and seroton during chronic manganese dioxide administra tion to squirrel monkeys. *Experientia* 196 25(11):1140-1141
Pakkenberg H, Brody H. The number of ner cells in the substantia nigra in paralysis agitan *Acta Neuropathol* 1965; 5:320-324
Papavasiliou PS, Miller ST, Cotzias GC, Kra HW, Hsieh RS. Sequential analysis: Mang nese, catecholamines and L-dopa induced dy kinesia. *J Neurochemistry* 1975; 25:215-219
Rinne, UK, Sonninen V. A double blind study L-dopa treatment in Parkinson's disease. *E Neurol* 1968; 1:180
Tang LC, Cotzias GC. L-3,4-dihydroxyphenyl lanine induced hypersensitivity simulating fo tures of denervation. *Proc Natl Acad Sci, US* 1977; 74:2126-2127

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Manganese and Extrapyramidal Disorders

(A Critical Review and Tribute to Dr. George C. Cotzias)

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ABSTRACT: In this essay we first review the important contributions of Dr. George Cotzias to the understanding of chronic manganese intoxication and of manganese metabolism in man and animals. We also indicate the original contribution of Dr. John Donaldson to the mechanism of the neurotoxicity of manganese. In a second phase, the author challenges the tenet that Parkinson's disease is a form of chronic manganese intoxication and that manganism is an experimental model for Parkinson's disease. Clinical, pathological, experimental and biochemical evidence are brought to bear on this argument. Thirdly the author proposes that the necessary event to the so-called "depigmentation" of the substantia nigra and subsequent bradykinetic "low dopamine" syndrome is an early enhanced turnover of dopamine. Manganese intoxication is only one of the factors which may serve as a trigger to this event. Many others are also listed. In opposition to current views, who look for causal factors in Parkinson's disease along the pathways for melanogenesis, the author thus proposes a novel hypothesis which envisions a variety of transient "trigger factors" acting at the dopamine synapse to increase dopamine turnover. In turn, this increased synthesis of dopamine favours the production of large quantities of free radicals within the cell bodies in the substantia nigra, eventually overflowing the scavenging capacity of neuromelanin and their protective barrier, and causing cell death. The resulting decreased pool of dopamine-producing cells leads to a self-perpetuating situation of ever increasing demand on the remaining cells, and "progression" of the disease. Finally the author stresses the fact that genetic factors may play a role in an individual's susceptibility to such triggers. Again defective manganese transport, metabolism or binding are only some of the mechanisms possibly underlying such genetic predisposition to induced basal ganglia disorders. Further studies relating to manganese in these disorders and particularly in Parkinson's disease should focus not on the "intoxication" part of the overload and its striato-

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pallidal consequences, but on the intimate mechanism of destabilization of the homeostatic regulator in neuromelanin bearing cells, even after the exposure period.

Key words: Manganese, Mechanism of Neurotoxicity, Substantia Nigra, Dopamine Turnover, Neuromelanin, Genetic Factors

INTRODUCTION

The present Symposium is organized in memory of one of the foremost medical scientists of our time, a man better known in the public for his practical accomplishments in the treatment of Parkinson's disease, but one who should be remembered by colleagues for his outstanding contributions to the understanding of the role of trace metals in the brain. George Cotzias personalized the very make-up of what a clinical scientist should be: first, an astute *clinician* who can, not only look at a variety of phenomena, but actually *see* the inconsistancies and paradoxes of the clinical picture and thus formulate intelligent questions to be answered; second a *scientist* who can imagine new ways to tackle the problem in experimental models, from simple, albeit original, working hypotheses; and third, a *physician*, who can leave the laboratory, and return with humanity to the patient, to try-out new therapeutic approaches based on his results and observations. George Cotzias was deservant of first place in all three parts of this trilogy.

From 1963 to his death, because of my own research, I had the occasion to follow closely everything that George published. As I will shortly demonstrate, our paths crossed at a number of points, and so occasionally did our swords, but always with the friendship of respect and the finality of honesty. During many of these exciting years, I had the pleasure of having in my department an extremely brilliant and capable biochemist who today is our chairman, John Donaldson. Together we followed a path sometimes parallel, sometimes quite different to that of Dr. Cotzias, but it is the purpose of the following presentation to illustrate how our ideas were in fact convergent, the end-result leading, we hope, to a better understanding of extrapyramidal disorders. Although you will find that my personal interpretation of the facts to be reviewed may differ from those of George Cotzias, or even of John Donaldson, the purpose of this presentation is to stimulate discussion, to challenge even when apparently criticizing. George Cotzias' friends will recognize in this his favorite method of confrontation.

PART ONE: A CONVERGENCE OF IDEAS

Cotzias and Manganese

Manganese, and its metabolism, was George Cotzias' first and everlasting leitmotiv in research: from 1953, and with a variety of collaborators, he pursued its investigation systematically, convinced as he was that in its mysteries lay the explanation for many of the clinical manifestations of extrapyramidal disorders. First he studied the partition of manganese among organs and organelles, to demonstrate its accumulation in the liver and particularly in mitochondria (Maynard and Cotzias, 1955) and thereafter the high specificity of its pathway in man, with absorption totally dependent on intake, and homeostatism regulated almost entirely by the rate of excretion in the bile and intestines (Borg and Cotzias, 1958; Cotzias and Greenough, 1958). In his first review of the problems associated with our understanding of manganese metabolism (Cotzias, 1958), Cotzias was able to outline some of the inconsistencies and questions that will challenge him and his collaborators for more than 20 years: manganese is an essential nutrient, and is apparently mainly involved in oxidation-reduction processes. While deficiency states appear to manifest themselves with ataxia, chronic poisoning is accompanied by extrapyramidal symptoms. It is at this moment (1958) that Cotzias stated for the first time that *Parkinsonism may be a form of chronic manganism*. With the availability of neutron activation analysis at Brookhaven, Cotzias and his colleagues had a powerful tool for the study of this problem (Papavasiliou and Cotzias, 1961; Cotzias, Miller and Edwards, 1966) and of the many interrelations between manganese and other trace elements, particularly magnesium, in normal and endocrinologically modified states (Cotzias, 1960; 1961; Hughes and Cotzias, 1961; Hughes, Miller and Cotzias, 1966). In this study, the authors made one of their first crucial and unexpected observations: while *in vitro* manganese could almost always replace magnesium, this was not at all the case *in vivo*. This dichotomy was reconciled by demonstrating that *in vivo manganese assumes valences higher than 2*, while magnesium does not. The living organism appears to be able to differentiate between these states. Consonant with this property is the behavior of radio manganese within the body. Thus it was found that only manganese can displace radiomanganese and that manganese turnover is totally dependent on intake. Its excretion is almost entirely fecal, while that of magnesium is through the kidney. This meant that *the pathway of manganese through the body is highly specific* and that, in some way, this important element is sequestered from the others (Cotzias, 1962; Hughes, Cotzias, and Cronkite, 1962; Cotzias and Papavasiliou, 1964; Bertinchamps, Miller and Cotzias, 1966).

Such observations inevitably led to the second series of major experiments: the investigation of binding mechanisms for manganese. The existence of more than one chemical species of manganese in the body was demonstrated (Cotzias and Papavasiliou, 1962). A protein was then discovered by Bertinchamps and Cotzias (1959), in human plasma, which binds manganese. It was called transmanganin, because, although a β_1 globulin, it was felt to be different from transferrin (the iron binding protein). Furthermore, Borg and Cotzias (1958a) brought forward evidence which supports the fact that mammalian and human red cells have one or more porphyrins which contain manganese instead of iron.

Cotzias and collaborators (Cotzias *et al*, 1961) had noted the clinical similarities between the extrapyramidal symptoms caused by phenothiazines and those of chronic manganese poisoning, and investigated the *effect of chlorpromazine on manganese binding*. It was shown (Borg and Cotzias, 1958b) that CPZ may compete for manganese with tissue ligands, thereby altering the distribution or availability of trace metals within cells. CPZ specifically combined *in vitro* with Mn^{+++}, and this was quenched by EDTA. A chromogenic reaction between phenothiazines and manganese was finally shown to be due to the formation of a semiquinone free radical ion in the chromophore (Borg and Cotzias, 1962a, 1962b). It was hypothesized from extrapolations of these experiments that one important physiologic role of cellular trace metals like manganese, may be *to serve in the formation of the free radical intermediates involved in oxidative metabolism* (Cotzias and Borg, 1962).

This observation led directly into the

third series of experiments. The aim of these studies was to discover whether manganese offered promise as a generator of free radicals in some other system of obvious biological significance. *Melanin* had been described as indeed being rich in free radicals. Hair, selected on the basis of its colour, was found to contain increasing concentrations of free radicals with increasing darkness of the specimens. Melanin was thus studied biochemically. It was shown (Cotzias, Papavasiliou and Miller, 1964) that dark structures of every individual examined contained much higher concentrations of manganese than did the white ones. In view of this finding *manganese was assigned to the final, auto-oxidative steps of melanogenesis* and a large series of investigations, particularly with Borg and Van Woert, led to the conclusion that melanosomes, or specific cytoplasmic organelles (Polymelanosomes), are to melanocytes what mitochondria are to non-pigmented cells: both contain large concentrations of manganese and free radicals (Prasad, Johnson and Cotzias, 1965; Van Woert, Nicholson and Cotzias, 1967).

In primates the greatest intensity of pigmentation is in the *substantia nigra* (Scherer, 1939). It is well known, of course, that severe depigmentation occurs in the substantia nigra, in the locus coeruleus and in the dorsal nucleus of the vagus in Parkinson's disease, but it is less well recognized that these areas are intact in albinos (Foley and Baxter, 1958). The cells of the substantia nigra appear to be melanocytes and to contain free radicals (Borg, 1974). Spectral studies of the pigment of the substantia nigra led to a better understanding of the biochemistry of neuromelamin (Van Woert, 1974; Van Woert and Ambani, 1974). Thus no tyrosinase is present in the substantia nigra. On the other hand, dopamine is easily auto-oxidized to form melanin. The semiquinone free radical of melanin can act as an electron acceptor. Finally, it was shown that melanin is also reactive with several drugs, particularly phenothiazine compounds. The *link between manganese, melanin, and the extrapyramidal system* had now been made, (Cotzias, 1966) and it was permissible to study the metabolism of this trace element in a variety of disorders of movement. This would be the next, and most important, phase of Dr. Cotzias's work.

The study of miners exposed to *chronic manganese overload* was the natural follow-up to these basic observations, particularly since many of the miners presented with extrapyramidal symptoms. This was accomplished with the cooperation of Chilean neurologists (Cotzias, Borg and Bertinchamps, 1960; Mena *et al*, 1967; Cotzias *et al*, 1968). It was shown that chronic manganese poisoning differs from Wilson's disease. Surprisingly tissue burdens of manganese were found to be higher in "healthy" miners than in ex-miners removed from the mines because of their extrapyramidal disease or mental changes ("locura manganica"). These symptoms included bradykinesia, postural difficulties, prominent rigidity, some tremor and occasionally significant dystonia. Turnover of radioactive manganese was significantly faster in "healthy" exposed miners than in the extrapyramidal patients or than in control normal subjects. Hair manganese concentration was only elevated in the "healthy" exposed miners. Thus these observations demonstrated that manganese overload was present only during exposure and was not in parallel to the presence of extrapyramidal symptoms. *It is thus not necessary to maintain high tissue levels of manganese to have neurological disease.* This capital, and unexpected, finding differentiated chronic manganese poisoning from Wilson's disease and, as now predicted, chelating agents were of no value in treatment. It was thus necessary to turn to other possible therapeutic approaches.

Melanocyte-stimulating hormone (β-MSH) is capable, in integumental melanocytes, of increasing melanin deposition (Lerner, Shizume and Bunding, 1954). This phenomenon is well-known in frogs. β-MSH can also reverse the effects of chlorpromazine (Krivoy and Guillemin, 1961). For these two reasons, β-MSH was tried in Parkinsonian patients in an effort to repigment the damaged substantia nigra (Cotzias, Van Woert and Schiffer, 1967). Unfortunately the Parkinsonian state was reversibly aggravated by the administration of this hormone and this approach was abandoned.

The L-Dopa Story

At this stage in our story it would be of interest to abandon Cotzias and his studies and to turn back a few years to my own laboratory to understand the background for the next important phase of the Cotzias saga. As I was completing my post-graduate studies in Montreal in 1956 and 1957, important developments were occuring in the understanding of biochemical events within the basal ganglia of the brain. Carlsson *et al.* (1958), Bertler and Rosengren (1959), as well as Sano and collaborators (1959) had demonstrated the presence of dopamine within the brain, and particularly within the basal ganglia. While training in neurology in Chicago in 1958, I had the pleasure of listening to Professor Carlsson lecturing at a meeting in Washington. At the time, I had been studying catecholamine excretion in various neurological disorders, and had found abnormalities in the urine of Parkinsonian patients (Barbeau, 1961). After that lecture, I decided to study the *specific* excretion of *dopamine* in Parkinson's disease. In these preliminary studies, as well as in more detailed later investigations in Professor Sourkes' laboratory in 1959 and 1960 (Barbeau, Murphy and Sourkes, 1961) we were able to clearly demonstrate a significantly decreased urinary excretion of dopamine in Parkinson's disease. It is of interest to note that while I was carrying out these experiments, one of my good friends, also training at the University of Chicago, was Dr. Melvin Van Woert, who was to play such an important role in the study of melanin in Dr. Cotzias' laboratory and who, throughout the years between 1959 and 1967, kept in touch with me and my DOPA studies.

At the same time and independently, Ehringer and Hornykiewicz (1960) reported similar dopamine deficits in the brain of Parkinsonian patients, particularly in the basal ganglia. It was thus natural for both our groups to attempt replacement therapy with dopamine, but this failed because of the blood-brain barrier to dopamine itself. We then turned to the natural precursor: L-DOPA. (Barbeau, 1961; Birkmayer and Hornykiewicz, 1961; Barbeau 1962), either intravenously or orally; following the crucial observations of Carlsson, Lindquist and Magnusson (1957) who had demonstrated that L-DOPA could reverse the extrapyramidal symptoms induced by reserpine. As is well-known, these early experiments in Parkinson's disease were successful and the physiological reversal of rigidity and akinesia in Parkinson's disease was clearly demonstrated. Although two similar studies were subsequently negative (McGeer and Zeldowicz, 1964; Fehling 1966), by 1968 the bulk of 32 publications were supportive of these findings (Barbeau, 1969). I had the privilege to present our own results with L-DOPA in Brookhaven, in Dr. Cotzias' department, on two occasions, in 1963 and 1966, and to discuss with him the therapeutic potential of this approach, which was then extremely expensive. By the second visit to Long Island, Dr. Cotzias had just completed his β-MSH studies and was postulating that the hormone was shifting

DOPA, the precursor of melanins and biogenic amines, from the brain to the integument. He was then attempting to treat Parkinsonian patients with progressively higher dosages of DL-DOPA.

This *stepwise, gradual, titration of L-DOPA* to very high levels, based on the biochemist approach to enzyme saturation, was the genial contribution of Cotzias and the key to the therapy of Parkinson's disease (Cotzias, Van Woert and Schiffer, 1967). The subsequent story of L-DOPA therapy, of its use in manganism, of its complications, of the improvements with DOPA decarboxylase inhibitors and dopa analogs, and the seminal role played by Cotzias and his collaborators in its development (Cotzias, Papavasiliou and Gellene, 1969; Cotzias, 1969; Mena *et al*, 1970; Cotzias *et al*, 1970; Cotzias *et al*, 1971) are well-known and have been detailed by me in a number of publications (Barbeau, 1969; Barbeau, 1976; Barbeau and Roy, 1976). I will therefore not review them here except to again stress their importance. However, it should be noted that the Brookhaven group was instrumental in demonstrating the important link existing between biogenic amines and manganese (Papavasiliou *et al*, 1975). This link could be 3',5' cyclic AMP. In sequential analyses, the authors demonstrated also significant correlations between levodopa-induced-dopamine dyskinesia and the concentration of dopamine and manganese. By studying a genetically abnormal species of mice, *pallid*, they showed that transportation of manganese and of L-DOPA was slower in these animals, and they demonstrated a remarkable resistance to the effects of orally or intraperitoneally administered L-DOPA (Cotzias *et al*, 1972). The *maturation and function of the dopamine apparatus may thus be manganese dependent*. Furthermore, genetically controlled metabolic predispositions (like in the pallid mouse) must be coupled with environmental factors before respective diseases can be induced.

Donaldson and Manganese

A third convergent series of experiments bearing on the present symposium must now be recounted, since they contribute new and important facts to the ideas we want to discuss. From 1968 to 1974, I was fortunate to have as a collaborator in my department, our chairman, Dr. John Donaldson. From the start, we decided that his field of research would be the study of trace metals in neurological disorders. The basis for this decision was a study we had just completed which confirmed the link between trace metals, phenothiazines and catecholamines first postulated by Cotzias and his group (Barbeau, 1968). Our first studies concerned the inhibition of NA^+-K^+-ATPase by different divalent cations and the production of convulsions or abnormal behaviors in animals receiving these metals intraventricularly (Donaldson *et al*, 1971; 1972; Izumi, Donaldson and Barbeau, 1973). This behavior resembled the effect of ouabain and enabled us to develop a working and reproducible model of epilepsy. (Donaldson, Minnich and Barbeau, 1972). We then used this model to test the role of certain trace metals (particularly zinc) and aminoacids in modifying this behavior (Izumi *et al*, 1973a, 1973b, 1973c). Taurine was found to be extremely effective in suppressing seizures (Barbeau and Donaldson, 1973; Tsukada *et al* 1974; Barbeau and Donaldson, 1974).

Zinc was not the only metal of interest to us. After determining in detail the distribution of various trace elements in rat brain regions (Donaldson, *et al*, 1973a), we studied the interaction of such elements upon cerebral monoamines (Barbeau *et al*, 1972, Donaldson *et al*, 1973b; Donaldson *et al*, 1974), demonstrating in passing that a deficiency in magnesium was generally accompanied by a brain dopamine deficit. At the time John Donaldson left us, we had just turned our

attention to manganese and its possible role in neurology and endocrinology, on the basis of the specific accumulation noted by him (Donaldson *et al*, 1973a) in the corpus striatum and in the hypothalamus. Injection of manganese into one caudate nucleus in rats resulted in a predominant ipsilateral turning behavior. At higher doses there were intermittent, alternating and dose-related contralateral turning resembling dystonia, and the production of stereotypies. Finally, bilateral injections produced severe bradykinesia (Inoue, Tsukada and Barbeau, 1975). The extremely high concentration of manganese in the median eminence of the hypothalamus led us to study the interaction between manganese and dopamine through its effect upon prolactin secretion. We clearly demonstrated that intraventricular manganese injections produced a dose dependent release of prolactin (Barbeau, Inoue and Cloutier, 1976).

Recently, John Donaldson, now in another laboratory, has pursued these leads in a series of extremely stimulating experiments, where he postulates that the possible basis for manganese neurotoxicity may be the enhanced autoxidation of dopamine by a higher valency manganese ion, with increased generation of free radicals and cytotoxicity (Donaldson, La Bella and Gesser, 1980; Donaldson, 1981). This hypothesis is supported in part by the studies of Graham and collaborators (Graham, 1978; Graham *et al*, 1978) demonstrating that manganese favors oxidation of dopamine to the corresponding semiquinone by Mn^{+++}, a reaction which would be followed by autoxidation. In more recent studies to be reported, Donaldson, McGregor and La Bella (unpublished) demonstrated almost complete abolition of lipid peroxidation in the striatum in acute manganese neurointoxication, thus a reduction of free radical activity, manganese being an effective free radical scavenger, either in its Mn^{++} form or in complexes such as superoxide dismutase (Kono, Takahashi and Asada, 1976; Archibald and Fridovich, 1981). It was thus postulated that endogenous manganese, in its Mn^{++} form, is normally a scavenger of free radicals and thus *a homeostatically controlled protector of the cells*. In certain areas with high concentrations of H_2O_2 and of oxidative enzymes like peroxidase, such as the substantia nigra (Cote and Fahn, 1979; Ambani, Van Woert and Murphy, 1975), peroxidase favours the oxidation of Mn^{++} to higher valency Mn^{++} or Mn^{+++} forms (Kenten and Maun, 1950). These forms, as shown above, favor the autoxidation of dopamine to semiquinone and the production of toxic free radicals ($OH^{\cdot}$, hydroxyl radicals). They, and the powerful oxidant Mn^{+++}, would be highly toxic to the cells where they are concentrated. Melanin containing cells appear to be the ideal milieu for such interactions because of their neurotransmitter and free radical make-up.

Conclusions

Following three different pathways, Cotzias, Donaldson and Barbeau have thus indicated that *a relationship exists between manganese, catecholamines and extrapyramidal disorders*. This observation is of utmost importance and is the major justification for the present symposium. However, despite the interest of these studies, there persists a great deal of confusion about the spontaneous extrapyramidal disease that manganese neurointoxication should be a model of: is it Parkinson's disease, or dystonia, or both? In the second part of this review we will address this most crucial point.

II: IS MANGANESE INTOXICATION A MODEL OF PARKINSON'S DISEASE?

Almost all previous authors, includ-

ing George Cotzias (Cotzias, 1958) have proceeded on the assumption that chronic manganese intoxication is indeed a form of Parkinsonism, despite normal levels of manganese in affected miners. From this assumption, the next theoretical step was easily taken, and it was soon proclaimed by some that manganese intoxication is a *model* of Parkinson's disease, and possibly even the *cause* of that disorder.

There is indeed an impressive array of facts and observations favouring such interpretations, but we would now like to challenge this conclusion on the basis of a critical review of the clinical, pathological, biochemical and therapeutic evidence presented in the literature. By so doing, we hope to generate new and innovative ideas for further research on the proper place of manganese in the pathogenesis of extrapyramidal disorders.

Clinical Evidence

The effects of chronic manganese intoxication in man have been known since the studies of Couper in 1837 (Couper 1837a,b) and have been described in many countries: Morocco, Chile, Cuba, India and the United States in particular. The clinical description is fairly uniform for the symptomatology in miners who are exposed to manganese dust inhalation, and has been reviewed in a multitude of papers and overviews (Casamajor, 1913; Seelert, 1913; Edsall, Wilbur and Drinker, 1919; von Oettingen, 1935; Rodier, 1950; Boyer and Rodier, 1954; Peñalver, 1955; Rodier 1955; Peñalver 1957; Cotzias, 1958; Murphy and Abbona, 1959; Cotzias, 1962; Wynter, 1962; Whitlock, Amuso and Bittenbender, 1966; Balani *et al*, 1967; Mena *et al*, 1967; Cotzias *et al*, 1968; Leach and Lilburn, 1978). Industrial manganese contamination, as particularly observed in the United States, is slightly different in presentation (Tanaka and Lieben, 1969; Greenhouse, 1971; Cook, Fahn and Brait, 1974). In both groups fatigue, somnolence, postural instability (particularly in the form of retropulsion and "démarche en pied de coq"), salivation, masked facies, bradykinesia and some mild rigidity are present. The differences reside in the absence of psychiatric prodroma (the "locura manganica") in industrial chronic manganese intoxication and the much more frequent dystonia in miners intoxicated by manganese dust inhalation.

In 1973, I was fortunate enough to visit Andacollo and the Corral Quemado Mines in Northern Chile with Dr. Jaime Court and to examine 10 patients suffering from the neurological complications of chronic manganese intoxication. These 10 patients are among the 13 studied in detail by Mena *et al*, (1970). Our examination, allowing for aging of the patients, confirms the observations made by these authors in 1967. However in studying further the "clumsiness in movements" and "increased tones", we were able to show that all 10 patients had increasing stiffness, to the point of blocking, with repeated movements. This appeared to be due to an inability to alternate contraction and decontraction in the opposing groups of muscles required for the specific repetitive movement. Further efforts always produced a dystonic posture of the limb, often accompanied by painful cramps (as in "writers cramps"). This attitudinal hypertonia had a tendency to decrease, or disappear, in the supine position and to increase in orthostation. The hypokinesia, certainly present in most of the patients, was not of the slow initiation type usual in Parkinson's disease, but resembled much more a positive blocking phenomenon, once repetitive movements were attempted. Moreover, in every one of the 10 patients, we could demonstrate at some point in time the "démarche en pied de coq" described by Seelert (1913). The patient uses small steps, but has a tendency to elevate the heels and to rotate them outward. He progresses without pressing on the flat of his feet, but only upon the metatarso-phalangeal articulations, mainly of the fourth and fifth toes. This posture may be absent upon initiation of walking, but it eventually appeared after a certain distance in every patient, unless he frequently stopped for rest.

Thus, our own personal observations in 10 cases of chronic manganese intoxication in chilean miners clearly indicate that some form of *dystonia* is an almost obligatory feature of the disease. It is our impression that reference to parkinsonism in such a situation is only valid if a comparison is made to post-encephalitic parkinsonism (as seen many years ago) but not to idiopathic Parkinson's disease. Moreover, the tremor observed in some of the patients is quite different from that seen in Parkinson's disease. It has much more of an attitudinal or flapping quality resembling that seen in Wilson's disease, or for that matter, in dystonia musculorum deformans (Johnson, Schwartz and Barbeau, 1962). However, cases of manganese intoxication from industrial exposure seem to be milder in presentation and much closer to idiopathic Parkinson's disease, particularly as far as the bradykinesia is concerned (Cook, Fahn and Brait, 1974).

In summary, the clinical evidence cannot be used to claim that chronic manganese intoxication is a form of Parkinsonism, except in the sense of the post-encephalitic model. It would be more accurate to state that this entity is a *model of extrapyramidal bradykinesia* (what we and Hornykiewicz elsewhere have called the "low dopamine syndrome") accompanied by a *dystonic syndrome of a striatal nature*. The appearance of this second syndrome appears to be related to the intensity of the exposure to manganese dust, particularly by inhalation, whereas the appearance of bradykinesia is more a factor of the duration of exposure, or perhaps more accurately, of the *length of time elapsed since exposure to a manganese load.*

Pathological Evidence

As is well known, the classical findings in Parkinson's disease are a *depigmentation* and *loss of cells* in the substantia nigra, locus coeruleus and dorsal nucleus of the vagus, often with the presence of inclusions called Lewy bodies, with little or no damage to the striatum or pallidum (Alvord *et al* 1974). The first description of the pathology of manganese poisoning is due to Casamajor in 1913, but Ashizawa (1927) was the first to properly describe the brain changes. He particularly emphasized the pallidal degeneration. Further studies were carried out by Flintzer (1931); Canavan, Cobb and Drinker (1934); Trendtel (1936); Voss (1939); Parnitzke and Peiffer (1954) and more recently by Bernheimer *et al* (1973). All authors found lesions evident in the pallidum, caudate nucleus and putamen. Only the latter authors reported depigmentation and cell damage in the substantia nigra. Thus a review of the accumulated pathological evidence indicates that the pallidum-subthalamic nucleus system may be preferentially damaged in manganese encephalopathy. Caudate nucleus and putamen are also constantly and severely involved. Involvement of the substantia nigra is probable, but is less well documented.

In summary, the pathological evidence is compatible with the clinical dichotomy and underlies the clinical admixture of dystonia and bradykinesia. This is *not* the classical pathology of idiopathic Parkinson's disease. Some would call it a "Parkinson-Plus" or more exactly a Multi-System disease of the brain which, incidentally, encroaches upon the substantia nigra.

Experimental Evidence

The experimental production of animal models of this disease confirms the same overall conclusions. The first experimental attempts at chronic manganese poisoning date from Kobert (1906)

in rabbits and von Jaksch (1907) in dogs, but both were unsuccessful. Lewy and Tiefenback (1921) administered powdered brownstone to rabbits over 3 months and observed abnormal postures. Mella (1923) injected manganese chloride i.p. to monkeys over 18 months and produced remarkable extrapyramidal symptoms of choreoathetosis followed by tremor, rigidity and extension of the fingers. At autopsy, the *putamen* and *caudate* showed widespread lesions. Grünstein and Popowa (1929) administered chronically 2 to 3 g. of manganese dioxide to rabbits and observed severe lesions in the *corpus striatum*, involving mainly the small cells. There was also marked glial cell proliferation in the same regions. Van Bogaert and Dallemagne (1943) carried out an extensive study of monkey brain poisoned with manganese. They were the first to use inhalation of manganese dioxide and were able to observe alternate extension and flexion of the upper limbs with spreading of the fingers and toes. The authors did not, however, observe a systematic atrophy of the pallidum, or of the striatum. There was some atrophy of the cerebellum and a diffuse atrophy of the grey matter of the anterior horns.

More recently Pentschew and collaborators (Pentschew, 1963; Pentschew, Ebner and Kovatch, 1963) gave a complete and detailed description of the neuropathology in monkeys intoxicated with manganese dioxide suspended in olive oil. Clear-cut dysfunctions of the extrapyramidal system, including dystonic postures, were seen and, at autopsy, the most severe lesions were in the *pallidum* and *subthalamic nucleus*. These lesions were said to be identical to those reported in the 3 cases of human manganese intoxication known to that date, and to involve mainly the pallidum.

Saxena (1967) and Chandra and Srivastava (1970) used manganese chloride i.p. daily in rats for a period of 180 days. Neuropathological examination performed at various intervals showed scattered neuronal degeneration in the cerebral and cerebellar cortex at 120 days which increased in intensity up to 180 days. The extent of the brain lesions was directly related to the amount of manganese in the brain tissue which increased with time. In these rats the basal ganglia did not reveal any pathological change.

It is of interest to note that Chandra and Tandon (1973) have shown that a deficiency in iron in rat leads to an increased accumulation and toxicity of manganese in the liver, kidney and testes. Histopathological changes were more pronounced in the liver and kidneys of iron deficient rats as compared to the findings in rats kept on a normal iron-containing diet. Finally Gupta, Murthy and Chandra (1980) demonstrated in monkeys muscular weakness and rigidity of the lower limbs after 18 months exposure to manganese chloride. For the first time, marked neuronal degeneration with gliosis, neuronal loss and depigmentation was noticed in the region of the substantia nigra. Neuromelanin in some cells was scanty when compared with normal pigmented cells and was displaced towards the periphery. The amount of manganese administered produced significant increases in the levels of the metal in the brain of these monkeys.

In summary, the experimental production of manganese intoxication in animals results in lesions that are much more diffuse than in Parkinson's disease. Although low dose *chronic* injections in primates can produce substantia nigra lesions, the damage is usually not confined to the brain stem, but can involve the striatum, the pallidum and even the cortex. Thus again we cannot say that manganese poisoning is a model of classical Parkinson's disease.

Biochemical Evidence: Behaviour of Manganese

Progress in this field has been directly parallel to the availability of exact methods of measurement of the trace quantities of manganese present in body fluids and tissues: neutron activation analysis (Papavasiliou and Cotzias, 1961; Cotzias, Miller and Edwards, 1966); atomic absorption spectroscopy (Suzuki and Wacker, 1974) and mass spectrometric identification (Hui, Davis and Boulton, 1979). A 24-hour rhythm in serum manganese levels was even demonstrated in rat (Schewing *et al*, 1968). In monkeys, Dastur, Manghani and Raghavendran (1971) studied the distribution and fate of ^{54}Mn. They showed tha t different organs, and different parts of the nervous system, behaved autonomously. The *lentiform* nucleus and *cerebellum*: exhibited marked relative retention in comparison to most viscera. It was suggested that the selective vulnerability of the brain in manganese miners might result from this inability on the part of the CNS to discharge the ^{54}Mn with time. However, Bull (1978) observed a paradoxical *decrease* in corpus striatum manganese concentrations after a manganese load ($MnCl_2$ in drinking water) for 6 months.

We should recall the seminal observations of Cotzias and his group (Cotzias, Borg and Bertinchamps, 1960; Mena *et al*, 1967; Cotzias *et al*, 1968; Papavasiliou *et al*, 1975) who clearly showed an increased turnover of ^{54}Mn in "healthy" exposed miners, but an apparently normal load in chronically affected miners removed from the mines. It would thus be of great interest to study actual manganese content in the brain of miners and in those of patients with Parkinson's disease. Unfortunately such observations are extremely rare. Larsen et al (1979) recently studied the topographical distribution of manganese and other metals in normal human brain by neutron activation analysis with radiochemical separation. Manganese seems to be associated with the dry matter of the brain. The distribution of manganese in the basal ganglia (with higher concentrations in the nucleus caudatus, globus pallidus and putamen than in the thalamus and substantia nigra) was consistently found despite some variations in absolute concentrations. This distribution confirms the findings which we had reported using atomic absorption spectroscopy (Barbeau, Inoue and Cloutier, 1976). The same group of authors (Larsen *et al*, 1981) recently studied manganese and selenium in two cases of Parkinson's disease. There were no obvious differences in the overall concentrations of these trace metals from the normal control group. Even multivariate data analysis failed to reveal any significant difference in the distribution pattern of manganese and selenium in Parkinson's disease compared to normal controls. Similar negative results concerning manganese were found by us (Barbeau and Cloutier, 1975, unpublished) in 3 cases of Parkinson's disease. These cases were not reported because we are still lacking proper age-matched and sex-matched normal controls.

On the other hand, elevated manganese concentrations have been found in some experimental and human conditions characterized by extrapyramidal symptoms: Bird and collaborators (Bird, Grant and Ellis, 1967; Bird *et al*, 1969) found *increased* manganese levels in the putamen and caudate nucleus of rhesus monkeys after phenothiazine treatments which produced dystonias, athetosis and some rigidity. Chronic administration of chlorpromazine resulted in increased levels of manganese in the caudate nucleus and cerebellar hemispheres in guinea pigs (Weiner, Nausieda and Klawans, 1977). These authors raised the possibility that tardive dyskinesia may somewhat be related to a manganese-dopamine interaction. In a later study (Weiner, Nausieda

nd Klawans, 1978), they showed that hronic administration of a levodopa-arbidopa combination, of bromocrip-ne or of lergotrile produced significantly ncreased concentrations of manganese in ll brain areas, with decreased copper oncentrations. It is well known, of course, hat chronic administration of these drugs an produce a variety of extrapyramidal anifestations. Recently, Banta and Markesberry (1977), described a patient vith Alzheimer dementia and extrapy-amidal signs (cogwheel rigidity, flexion osturing, bradykinesia, retropulsion, nasked facies) who had markedly ele-ated levels of manganese in serum, hair, rine, feces and brain.

In summary, the evidence gathered rom the determination of manganese evels and turnover rates in brain and body fluids in idiopathic Parkinson's dis-ase does not support the idea that such a lisease is accompanied by *chronically levated* manganese concentrations. Nor s this the case in experimental models. In uch models, when manganese concen-ration is high, *hyper*kinetic rather than *hypo*kinetic symptoms are present. How-ver, it would be worthwhile to gather much more information on this point hrough the use of hair and blood deter-minations of manganese (Laker, 1982) in large number of well-defined extrapy-ramidal disorders and age-matched nor-mal controls.

Biochemical Evidence: The Behaviour of Catecholamines

One of the potent arguments in favour of the similarity between Parkinson's dis-ease and chronic manganese intoxication has been the interaction noted between this metal and catecholamines and the biochemical resemblance of the dopa-mine depletion. This has been studied by a number of authors since Papavasiliou, Miller and Cotzias (1968) demonstrated a functional interaction between biogenic amines, cyclic AMP and manganese. The following year, Neff, Barrett and Costa (1969) showed a selective depletion of caudate nucleus dopamine and serotonin during chronic manganese dioxide admin-istration to squirrel monkeys ($Mn\ O_2$ ground in a mortar with olive oil added). Norepinephrine concentrations were not affected. Mustafa and Chandra (1971) confirmed the dopamine deficit in inocu-lated rabbits, but they reported normal levels of serotonin and decreased concen-trations of norepinephrine. Pursuing these leads, Prasad (1974) demonstrated that excess of manganese may markedly reduce the intracellular level of cAMP by inhibit-ing adenylate cyclase activity and stimu-lating phosphodiesterase activity. Man-ganese, when incorporated, appears to bind to ATP and to displace catechola-mines in chromaffin granules (Daniels, Johnson and Williams 1979). Bonilla and Diez-Ewald (1974) then showed that rats chronically treated with a high oral load of $MnCl_2$ have decreased concentrations of dopamine and HVA in the brain. A return to normal values was observed after L-DOPA injections. This suggested that the disturbance could be at the L-Tyrosine hydroxylase step (Bonilla, 1974). However, not every author could confirm these reports. Bull (1978) failed to find a decrease in corpus striatum dopamine after a manganese load, using a protocole similar to that of Bonilla and Diez-Ewald (1974). Kimura, Yagi and Itokawa (1978) found lower serotonin concentrations after subacute manganese feeding in Wistar rats. They also reported lower values of L-aromatic amino acid decar-boxylase activity. Manganese levels were elevated in tissues and this was accom-panied by lower magnesium and calcium levels in the brainstem.

In further studies, Bonilla and his collaborators (Bonilla, 1978; 1980; Mar-tinez and Bonilla, 1981) reported an increased GABA content in the caudate nucleus of rats; values for L-Tyrosine hydroxylase in neostriatum, midbrain and hippocampus, were elevated for the first 3 months of manganese intoxication, then decreased significantly in the neostriatum but not in the other regions studied. Ace-tylcholinestrase activity diminished in the caudate nucleus only in the 8th month of intoxication but was not altered in any other region. However, Deskin, Bursian and Edens (1980) showed *in vitro* that the addition of manganese to brain prepara-tions had no direct effect on tyrosine hydroxylase activity and that manganese does not compete with other physiologi-cally important divalent cations to cause the reduction in tyrosine hydroxylase activity seen *in vivo* after chronic exposure.

These observations lead to crucial experiments by Shukla and Chandra (1981) and by Lai and collaborators (1982). The first authors investigated the turnover rate of striatal dopamine after the daily oral administration of $Mn\ Cl_2.4H_2O$ for 30 days. The *turnover rate of striatal dopamine*, as determined by the administration of α-methyl-p-tyrosine and measurement of HVA, *increased sig-nificantly* in the early phase of manganese intoxication in rats. Similarly Lai *et al* (1982) observed a decrease in the uptake of dopamine in rats by hypothalamic synaptosomes. This decrease was age-dependent for it disappeared after 100 days of manganese treatment. These most important observations recall similar evidence for peripheral increases in dopa-mine turnover in the early phases of human Parkinson's disease which we had previously published (Barbeau, 1968; Barbeau and Trombitas, 1969; Montplai-sir and Barbeau, 1969). In further exper-iments (Lai, Leung and Lim, 1981, 1982; Lai *et al.*, 1981; Leung, Lai and Lim, 1981), it was shown that life-long expo-sure to high manganese levels appeared to abolish the age-related decreases in ace-tylcholinesterase, in glutamic acid decar-boxylase, in NAD-linked isocitric dehy-drogenase and in choline acetyl transferase activities. If these important findings can be confirmed by others, the role of man-ganese in the prevention of aging changes will have to be considered seriously.

In summary, experimental chronic manganese poisoning appears to be accompanied by decreases in the concen-tration of dopamine in the caudate nucleus, but this seems to be age and spe-cies dependent. No direct correlation has yet been made with the state of pigmenta-tion of the substantia nigra.

Conclusions

The accumulated evidence reviewed above, be it clinical, pathological, exper-imental or biochemical, indicates that Parkinson's disease in its idiopathic form, and chronic manganese intoxication, *are not* identical entities. Statements such as "Parkinson's disease is a form of chronic manganese poisoning" are therefore no longer valid. High manganese concentra-tions in the striatum are associated with extrapyramidal dyskinesias of the *hyper-kinetic* type. Only the bradykinesia of low level chronic manganese intoxication, with its accompanying low striatal dopamine concentration and substantia nigra depig-mentation, can be correlated with what is seen in Parkinson's disease. But it should always be remembered that this brady-kinesia *is only one part*, and not the most important one, of the clinical picture of chronic manganism, which is a much more complex multi-system disease.

Nevertheless the numerous similari-ties are still intriguing. Manganese could still play a role in Parkinson's disease. This is what we will analyze in the third part of this essay.

III. WHAT IS THE ROLE OF MANGANESE IN PARKINSON'S DISEASE?

In the above two parts we reviewed

he three different pathways followed by otzias, Donaldson and Barbeau to reach very similar general conclusion: that anganese has a role to play in the athogenesis of some extrapyramidal isorders. Most of the landmark observaions and experiments have been made by eorge Cotzias and his collaborators, while John Donaldson improved our understanding of the mode of action of manganese with his demonstration of the facilitation by this trace element of the autoxidation of dopamine and the enhanced production of free radicals, which an increase in manganese valency can facilitate. Unfortunately my own contribution is more esoteric, with a direct challenge at the basic tenet of the previous publications: that parkinsonism is a form of chronic manganese poisoning. The evidence, as reviewed by me, indicates rather that the clinical picture of acute and sub-acute manganese intoxication is more akin to dystonia than to parkinsonian rigidity. The observed pathology in the striatum and pallidum is mainly compatible with the clinical picture of hyperkinetic dyskinesia. Finally, the experimental feeding of manganese to rats and monkeys results in dyskinesia and pathological lesions in the striatum and pallidum, lesions which are not usually encountered in Parkinson's disease. On the other hand, there remains one very important similarity between the two entities: the substantia nigra can be depigmented and dopamine concentrations in the striatum can be decreased when exposure to manganese is of long duration. Why then is there still confusion about the role of manganese in basal ganglia diseases? In the following pages, I will express my own interpretation of the above noted facts and observations.

A Few Wrong Assumptions:

1. The term *"basal ganglia"* is not always utilized in its proper connotation. It should never be used as reflecting a homogeneous nucleus. Disorders where the lesion is in the caudate nucleus are not necessarily the same as those affecting the pallidum or the substantia nigra. This truism is unfortunately too often neglected by non-clinical scientists. Dystonia musculorum deformans, Wilson's disease, Huntington's chorea and Parkinson's disease are all disorders of the "basal ganglia", but their clinical presentation is quite different. It would thus be very improbable for one animal experimental model to represent all of these entities at once.

2. The *substantia nigra "depigmentation"* is often though of as representing a decrease in the pigmentation of cells within that nucleus. In fact, this paller appearance results from death of a number of cells with loss of their melanin pigments. These are released outside the remnants of the cell and phagocytosed by glial cells who carry them to neighbouring capillaries. One must therefore search for the cause of cell death or fragility rather than try to unravel phenomena which would "demelanize" the substantia nigra cells. It seems to me that the loss of the pigmented cells *precedes* the decrease in striatal dopamine and not that some defect in dopamine synthesis results in incomplete melanogenesis (along the model of what occurs to tyrosinase in albinism in the integuments). If that is so, one should study the role of melanin pigments in the *homeostatism* of these specialized cells and investigate what happens to his homeostatism in aging, in Parkinson's disease and in chronic manganese poisoning. That dopamine is important in melanogenesis cannot be denied (Garcia-Carmona *et al*, 1981); that it is the neurotransmitter most affected is manganese intoxication is also a fact (Cotzias *et al* 1976). What is still uncertain, is the sequence in which such events occur. Determining that sequence is of the utmost importance to understanding the role of manganese in basal ganglia diseases.

Some Important Deductions

1. *The role of melanin.* Contrary to lipofuscin, neuromelanin in substantia nigra, locus coeruleus and dorsal nucleus of the vagus is not an aging pigment. It is a *homeostatic regulator of free radicals.* In other words it protects certain specialized cells from the accumulation within their inner space of toxic substances, like free radicals, by scavenging them. Otherwise the cell membranes, or more probably the organelle membranes, would be damaged, leading to cell death. This occurs mainly in cells which produce catecholamines (dopamine and noradrenaline), because these are the neurotransmitters most susceptible to autoxidation. Neuromelanin is thus a controller, or regulator, of dopamine cell growth and maintenance. Neuromelanin accumulation increases with phylogeny and is most important in primates where the dopamine pathways are most active. In this context, "activity" means turnover rate as much as spread of the network. Whenever turnover rate ("activity") of dopamine exceeds the capacity of the melanin pigments to scavenge the free radicals produced from dopamine, these toxic substances accumulate within the cells and damage their energy mechanism and their structure. Cell death may result and then phagocytosis of the abandoned pigments. In my view, therefore, neuromelanin is a necessary protector of substantia nigra cells. Because of the very nature of these products, it is only when one "overloads" the protective mechanisms of the cells that disease results, not when the pigment itself is sick.

2. *Consequence of nigral "depigmentation"*: when substantia nigra cells die and lose their melanin pigments, the nigro-striatal fibers originating from that nucleus and reaching the striatum also disappear. Since dopamine is produced in these cells and liberated in the caudate and putamen, there results a decrease in the concentration of dopamine in the striatum. Clinically the manifestation of this "low dopamine syndrome" is bradykinesia. This symptom cannot be equated to Parkinson's disease. It is part of that disorder, but it is also present in other entities: Shy-Drager, some olivo-pontocerebellar atrophies, and chronic manganism, particularly after long exposure. At no time does such a sequence of events result in the pathological lesions observed in the striatum and pallidum in manganese poisoning. Other events must explain these lesions.

3. *The lesions in the strio-pallidum*: these lesions are the only ones that can explain the hyperkinetic dyskinesias observed in manganese intoxication. For some reason, trace elements tend to accumulate within these nuclei. This is true for iron in Hallervorden-Spatz disease and for copper in Wilson's disease. It is also a fact for manganese after exposure to the metal or after chlorpromazine. It is this very accumulation of the divalent cations which destabilizes trace element homeostatism within the cells, probably by displacement, and eventually kills them. We have seen above that manganese blocks cAMP formation in post-synaptic regions, thus interrupting the chain of neurotransmission. Similarly, Meiri and Rahamimoff (1974) have shown that manganese decreases reversibly the amount of transmitter liberated presynaptically by nerve impulse. All these phenomena are compatible with the dystonia and abnormal postures observed during the "overload" period of manganese intoxication, a period during which ^{54}Mn turnover as well as dopamine turnover have been found to be more rapid. A similar mechanism within the dopaminergic meso-limbic system could suffice to explain the early psychotic episodes

("locura manganica"). However none of these direct actions can explain what occurs in the substantia nigra, nor the clinical accompaniment of bradykinesia. Unless major cell damage has been perpetuated in the striatum and pallidum, the symptoms of dystonia are reversible with time, after removal from the source of exposure.

4. *The lesions in the substantia nigra.* These are the most important for those of us trying to understand idiopathic Parkinson's disease. I would like to propose a new model for their pathogenesis, based on our understanding of the role of melanogenesis in the substantia nigra (see above). The necessary event is an *increased turnover of dopamine*. Once this occurs, *autoxidation of the amine increases* and *free radicals* are produced in quantity within cell bodies, eventually overflowing the capacity of melanin to scavenge them (binding to render non toxic). The free radicals then *kill the cells*. Melanin is released, phagocytosed and removed from the scene where gliosis follows. As a consequence, nigro-striatal fibers decrease in number, less dopamine can be liberated within the striatum and bradykinesia appears. The dopamine receptors in the striatum become supersensitive (denervation supersensitivity) and react to exogenous inputs of dopamine or agonists with the production of dyskinesia. Most of that sequence was well known; what is original is the need for an *increase in dopamine turnover as a trigger event* to this sequence. This increased turnover has indeed been noted in manganese intoxication (Shukla and Chandra, 1981; Lai *et al*, 1982), in phenothiazine intoxication (Barbeau 1968) and in early Parkinson's disease (Barbeau, 1968, Barbeau and Trombitas, 1969). It is important to stress that this is a *transient event*, always occuring very early in the process. It does not have to continue throughout the duration of the illness, once *destabilization of the regulatory homeostatism* in the substantia nigra has taken place. It is thus veritably a *trigger event*.

5. *The site of the trigger process:* to increase dopamine turnover, it is necessary to functionally block the transmission process at the synapse or to disregulate the feedback information mechanism. This can be accomplished at the post-synaptic level by interfering with the receptor sites (Haloperidol acts there), or the second messenger mechanism (manganese blocks cAMP formation) or at pre-synaptic sites by blocking release of the transmitter (manganese). Whenever such a block occurs, dopamine turnover will increase in order to overcome the block. This means a considerable demand on the synthetic machinery in the cell bodies.

In idiopathic Parkinson's disease, one does not, of course, know the nature of the *trigger event* to early *increased dopamine turnover*. It could be the use of phenothiazines or other neuroleptics to treat psychiatric disturbances; it could be hormonal influences (like thyroid hormone or estrogens) modifying the reactivity of the dopamine receptors; it could be the use of amphetamines (dopamine releasers) in reducing diets; it could be organ-specific or receptor specific, autoimmune reactions; it could even be a transient exposure to larger amounts of a trace metal like manganese. As far as I am concerned, what ever the *trigger event*, it should act preferentially at synaptic nerve endings, not at the level of cell bodies. For our discussion, this means that the trigger event acts in the striatum or limbic system, not in the substantia nigra itself.

6. *The role of genetic predispositions*: There is now growing evidence (Barbeau and Pourcher, 1982; Roy, Boyer and Barbeau, 1982) that genetic factors play an important role in Parkinson's disease and even in the reaction of individuals to neuroleptics. We have also clearly identified familial sub-groups of Parkinson's disease. Such genetic predisposition could be manifested by anomalies at one or more of three different levels: 1) at the post-synaptic membrane, where an anomaly could occur at the structural level (proteins) or in the biochemical mechanism controlling secondary transmission (for example a defect in the adenylate cyclase mechanism or in phospholipid or methionine metabolism); 2) in the metabolism of dopamine itself (particularly the methylating mechanism with formation of abnormal methylated metabolites); 3) in the substantia nigra where dopamine is produced. In the latter site an anomaly could occur in the composition of the scavenging mechanism in melanin. For example the binding of manganese to a specific protein or as a constituant metalloprotein like superoxide dismutase (Keele, McCord and Fridovich, 1970; Scruton, 1971; Foradori and Dinamarca, 1972; Weisiger and Fridovich, 1973; Bonilla, 1977; Vanella *et al*, 1982) could be deficient or abnormal, leading to easier escalation of the valency range from Mn^{++} to Mn^{+++} and to more toxicity when this ion combines with available free radicals, as was proposed by Donaldson and Graham (see above). It is interesting that mitochondrial superoxide dismutase (which is a manganese form) was shown to increase in concentration with normal aging (Vanella *et al*, 1982).

All these possibilities must be studied systematically before any firm conclusion can be drawn, but it is evident that a genetic susceptibility may exist in some patients to the production of an increased dopamine turnover, or to the detoxification of toxic substances in the substantia nigra. Once a *trigger event* has initiated the process, nigro-striatal degeneration will take place and will be a self-perpetuating phenomenon because of the ever decreasing pool of dopamine-producting cells, with the eventual clinical production of bradykinesia. In this respect, it is interesting to note that in familial juvenile Parkinsonism where the disease starts early, a probable congenital absence of neuromelanin in the substantia nigra has recently been reported (Narabayashi, 1981).

CONCLUSIONS

1. In this essay we first review the important contributions of Dr. George Cotzias to the understanding of chronic manganese intoxication and of manganese metabolism in man and animals. We also indicate the original contribution of Dr. John Donaldson to the mechanism of the neurotoxicity of manganese.

2. In a second phase, the author challenges the tenet that Parkinson's disease is a form of chronic manganese intoxication and that manganism is an experimental model for Parkinson's disease. Clinical, pathological, experimental and biochemical evidence are brought to bear on this argument.

3. Thirdly, the author proposes that the *necessary event* to the so-called "depigmentation" of the substantia nigra and subsequent bradykinetic "low dopamine" syndrome is an early enhanced turnover of dopamine. Manganese intoxication is only one of the factors which may serve as a trigger to this event. Many others are also listed.

4. In opposition to current views, who look for casual factors in Parkinson's disease along the pathways for melanogenesis, the author thus proposes a novel hypothesis which envisions a variety of transient "trigger factors" acting at the dopamine synapse to increase dopamine turnover. In turn, this increased synthesis of dopamine favours the production of large quantities of free radicals within the cell bodies in the substantia nigra, eventually overflowing the scavenging capacity of neuromelanin and their protective barrier and causing cell

death. The resulting decreased pool of dopamine-producing cells leads to a self-perpetuating situation of ever increasing demand on the remaining cells, and "progression" of the disease.

5. Finally the author stresses the fact that genetic factors may play a role in an individual's susceptibility to such triggers. Again defective manganese transport, metabolism or binding are only some of the mechanisms possibly underlying such genetic predisposition to induced basal ganglia disorders.

6. Further studies relating to manganese in these disorders and particularly in Parkinson's disease should focus not on the "intoxication" part of the overload and its striato-pallidal consequences, but on the intimate mechanism of destabilization of the homeostatic regulator in neuromelanin bearing cells, even after the exposure period.

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REFERENCES

Alvord EC, Forno LS, Kusske JA, Kaufman RJ, Rhodes, JS and Goetowski CR. The pathology of Parkinsonism: a comparison of degerations in cerebral cortex and brainstem. *Adv in Neurol* 1974; 5:175-193

Ambani LM, Van Woert MH and Murphy S. Brain peroxidase and catalase in Parkinson disease. *Arch Neurol* 1975; 32:114-118

Archibald FS and Fridovich I. Manganese, superoxide dismutase and oxygen tolerance in some lactic acid bacteria. *J Bact* 1981; 146:928-936

Ashizawa R. Ueber einen sektionsfall von chronischer manganvergiftung. *Jap J Med Scien Trans VIII Int Med Pediat & Psychiat* 1927; 1:173-191

Balani SG, Umarji GM, Bellare RA and Merchant HC. Chronic Manganese Poisoning. *J Postgrad Med* 1967; 116-122

Banta RG and Markesbery WR. Elevated manganese levels associated with dementia and extrapyramidal signs. *Neurology* 1977; 27:213-216

Barbeau A. Dopamine and basal ganglia diseases. *Arch Neurol* 1961a; 4:97-102

Barbeau A. Biochemistry of Parkinson's Disease. *Excerpta Medica* 1961b; ICS 38:152-153

Barbeau A. The pathogenesis of Parkinson's Disease: a new hypothesis. *Can Med Ass J* 1962; 87:802-807

Barbeau A. Dopamine and dopamine metabolites in Parkinson's disease -- a review. *Proc Austral Ass Neurol* 1968a; 5:95-100

Barbeau A. Effect of Phenothiazines on dopamine metabolism and biochemistry of Parkinson's Disease. *Agressologie* 1968b; 9:3-8

Barbeau A. L-DOPA therapy in Parkinson's Disease: a critical review of nine years' experience. *Can Med Ass J* 1969; 101:791-800

Barbeau A. Six years of high level Levodopa therapy in severely akinetic parkinsonian patients. *Arch of Neurol* 1976; 33:333-338

Barbeau A and Donaldson J. Taurine in Epilepsy. *The Lancet* 1973; 2:387

Barbeau A and Donaldson J. Zinc, Taurine and Epilepsy. *Arch of Neurol* 1974; 30:52-28

Barbeau A, Inoue N and Cloutier T. Role of Manganese in Dystonia. *Advances in Neurol* 1976; 14:339-352

Barbeau A, Murphy GF and Sourkes TL. Excretion of dopamine in diseases of basal ganglia. *Science* 1961; 133:1706-1707

Barbeau A and Pourcher E. New data on the genetics of Parkinson's Disease. *Can J Neurol Sci* 1982; 9:53-60

Barbeau A, Rojo-Ortega JM, Brecht HM, Donaldson J, Minnich J, and Genest J. Effect of a magnesium-deficient diet on the striatal content of amines in the dog. *Experientia* 1972; 28:289-290

Barbeau A and Roy M. Six-year results of treatment with Levodopa plus Benzerazide in Parkinson's Disease *Neurology* 1976; 26:399-404

Barbeau A and Trombitas S. The metabolism of tritium labelled dopamine in Parkinsonian patients. In: *Progress in Neurogenetics.* Barbeau A and Brunette JR, eds. Excerpta Medica Foundation, Amsterdam, 1969; pp 352-356

Bernheimer H, Birkmayer W, Hornykiewicz O, Jellinger K and Seitelberger F. Brain dopamine and the syndromes of Parkinson and Huntington — clinical, morphological and neurochemical correlations. *J Neurol Sci* 1973; 20:415-425

Bertler A and Rosengren E. Occurence and distribution of catecholamines in brain. *Acta Physiol Scand* 1959; 47:350-355

Bertinchamps AJ and Cotzias GC. A new manganese binding protein. *Fed Proc* 1959; 18:469

Bertinchamps AJ, Miller ST and Cotzias GC. Interdependence of routes excreting manganese. *Amer J Physiol* 1966; 211:217-224

Bird ED, Collins GH, Dodson MH and Grant LG. The effect of Phenothiazine on the manganese concentration in the basal ganglia of subhuman primates. In: *Progress in Neuro-genetics.* Barbeau A and Brunette JR eds. Excerpta Med Found, Amsterdam 1969; pp 600-605

Bird ED, Grant LG and Ellis WH. Measurement of the effect of phenothiazine on the manganese concentration in the basal ganglia of subhuman primates by activation analysis. In: *Nuclear Activation Techniques in the Life Sciences.* IAFA, Vienna, 1967; pp 491-499

Birkmayer W and Hornykiewicz O. Der L-3-4-dioxyphenylalanin (=Dopa) effect bei der Parkinson-akinese. *Wien Klin Wschr* 1961; 45:878-888

Bonilla E. Estudios *in vivo* e *in vitro* del metabolismo del manganeso. Manganismo cronico experimental y catecolaminas. *Investigacion Clinica* 1974; 15:2-79

Bonilla E. Isolation of a manganese binding protein from mouse liver. *Investigacion Clinica* 1977; 18: 197-203

Bonilla, E. Increased GABA content in caudate nucleus of rats after chronic manganese chloride administration. *J Neurochem 1978; 31: 551-552*

Bonilla E. L-Tyrosine hydroxylase activity in the rat brain after chronic oral administration of manganese chloride. *Neurobehavioral Toxicol* 1980; 2:37-41

Bonilla E and Diez-Ewald M. Effect of L-DOPA on brain concentration of dopamine and homovanillic acid in rats after chronic manganese chloride administration. *J Neurochem* 1974; 22:297-299

Borg DC. Free radicals in the human nervous system. *Advances in Neurology* 1974; 5:277-286

Borg DC and Cotzias GC. Incorporation of manganese into erythrocytes as evidence for a manganese-porphyrin in man. *Nature* 1958a; 182:1677-1678

Borg DC and Cotzias GC. Binding of manganese by homogenized mouse brain and the effect of chlorpromazine. *Fed Proc* 1958b; 17:430

Borg DC and Cotzias GC. Manganese metabolism in man: rapid exchange on Mn^{56} with tissue as demonstrated by blood clearance and liver uptake. *J Clin Invest* 1958c; 37:1269-1278

Borg DC and Cotzias GC. Interaction of trace metals with phenothiazine drug derivatives. I. Structure-reactivity correlations. *Proc Nat Acad Sci* 1962a; 48:617-623

Borg, DC and Cotzias GC. Interaction of trace metals with Phenothiazine drug derivatives. II. Formation of free radicals. *Proc Nat Acad Sci* 1962b; 48:623-642

Boyer J and Rodier J. Aspects neurologiques de l'intoxication professionnelle par le manganèse. *Rev Neurol* 1954; 90:13-27

Britton AA and Cotzias GC. Dependence of manganese turnover on intake. *Amer J Physiol* 1966; 211:203-206

Bull RJ. Paradoxical decrease in corpus striatal manganese concentrations with manganese load. *Comm in Psychopharmacol* 1978; 2:17-20

Canavan M, Cobb S and Drinker CK. Chronic manganese poisoning - report of a case with autopsy. *Arch Neurol Psychiat* 1934; 32:501-512

Carlsson A, Lindqvist M and Magnusson T. 3,4-Dihydroxyphenylalanine and 5-hydroxytryptophan as reserpine antagonists. *Nature* 1957; 180: 1200-1201

Carlsson A, Lindqvist M, Magnusson T and Waldeck B. On the presence of 3-hydroxytyramine in brain. *Science* 1958; 127:471

Casamajor L. An unusual form of mineral poisoning affecting the nervous system: manganese? *J Amer Med Ass* 1913; 60:646-649

Chandra SV and Tandon SK. Enhanced manganese toxicity in iron-deficient rats. *Environ Physiol Biochem* 1973; 3:230-235

Chandra, SV and Srivastava SP Experimental production of early brain lesions in rats by parenteral administration of manganese chloride. *Acta Pharmacol et Toxicol* 1970; 28:177-183

Cook DG, Fahn S and Brait KA. Chronic manganese intoxication. *Arch Neurol* 1974; 30:59-64

Cote IJ and Fahn S. Some aspects of the biochemistry of the substantia nigra of the thesus monkey. In: *Progress in Neuro-genetics.* Barbeau A and Brunette JR, eds. Excerpta Medica Foundation, .hw Am-ster-dam 1969; pp 311-317

Cotzias GC. Manganese in health and disease. *Physiol Reviews* 1958; 38:503-532

Cotzias GC. Metabolic relations of manganese to other minerals. *Fed Proc* 1960; 19:655-658

Cotzias GC. Manganese, versus magnesium: why are they so similar *in vitro* and so different *in vivo*? *Fed Proc* 1961; 20:98-103

Cotzias GC. Manganese. In: *Mineral Metabolism: an Advanced Treatise.* Comar CL and Bronner F, eds. Academic Press Inc., New York, New York 1962; 28:403-442

Cotzias GC. Manganese, Melanins and the Extrapyramidal system. *J Neurosurgery* (Suppl.) 1966; 24:170-180

Cotzias GC. Metabolic modification of some neurologic disorders. *J Amer Med Assoc* 1969; 210:1255-1262

Cotzias GC and Borg DC. Phenothiazines and manganese. In: *Ultrastructure and Metabolism of the Nervous System* (A.R.N.M.D.) 1962; Vol XI. pp 337-357

Cotzias GC, Borg DC and Bertinchamps AI. Clinical experiences with manganese. In: *Metal Binding in Medicine*. Seven MJ and Johnson LA, eds. Philadelphia, JB Lippincott Co 1960; pp 50-57

Cotzias GC, Borg DC, Hughes ER, Bertinchamps A and Papavasiliou P. Phenothiazines: curative or causative in regard to Parkinsonism? *Rev Can Biol* 1961; 20:289-294

Cotzias GC and Greenough JJ. The high specificity of the manganese pathway through the body. *J Clin Invest* 1958; 37:1298-1305

Cotzias GC, Horiuchi K, Fuenzalida S and Mena I. Chronic manganese poisoning - clearance of tissue manganese concentrations with persistence of the neurological picture. *Neurology* (Minn) 1968; 18:376-382. 1969; pp 600-605

Cotzias GC, Miller ST, Papavasiliou PS and Tang LC. Interactions between manganese and brain dopamine. *Med Clin North Amer* 1976; 60:729-738

Cotzias GC and Papavasiliou PS. State of binding of natural manganese in human cerebro-spinal fluid, blood and plasma. *Nature* 1962; 195:823-824

Cotzias GC and Papavasiliou PS. Primordial homeostasis in a mammal as shown by the control of manganese. *Nature* 1964; 201:828-829

Cotzias GC, Papavasiliou PS, Fehling C, Kaufman B and Mena I. Modification of Parkinsonism with dopamine receptor agonists. *New England J Med* 1970; 282:31-33

Cotzias GC, Papavasiliou PS and Gellene R. Modification of Parkinsonism - Chronic treatment with L-DOPA. *New Engl J Med* 1969; 280:337-345

Cotzias GC, Papavasiliou PS, Ginos J, Steck A and Duby S. Metabolic modification of Parkinson's disease and of chronic manganese poisoning. *Ann Rev Medicine* 1971; 22:305-326

Cotzias GC, Papavasiliou PS, Mena I, Tang LC and Miller ST. Manganese and catecholamines in McDowell FH and Barbeau A, eds. *Advances in Neurology* 1974; 5:235-243

Cotzias GC, Papavasiliou PS and Miller ST. Manganese in melanin. *Nature* 1964; 201:12281229

Cotzias GC, Tang LC, Miller ST, Sladic-Simic D and Hurley LS. A mutation influencing the transportation of manganese, L-DOPA, and LTryptophan. *Science* 1972; 176: 410-412

Cotzias GC, Van Woert MH and Schiffer LM. Aromatic amino acids and modification of Parkinsonism. *New Engl J Med* 1967; 276:374-379

Couper J. Sur les effets du peroxide de manganese. *J Dr Chim Med de Pharm et de Toxicol* 1837a; 3: 223-225

Couper J. On the effects of black oxide of manganese when inhaled into the lungs. *Br Ann Med Pharmacol* 1837b; 1:41-42

Daniels AJ, Johnson LN and Williams RJP. Uptake of manganese by chromaffin granules *in vitro J Neurochem* 1979; 33:923-929

Dastur DK, Manghani DK and Rachavendras KV. Distribution and fate of ^{54}Mn in the monkey: studies of different parts of the central nervous system and other organs. *J Clinical Invest* 1971; 50:9-20

Deskin R, Bursian SJ and Edens FW. An investigation into the effects of manganese and other divalent cations on tyrosine hydroxylase activity. *Neurotoxicology* 1980; 2:75-81

Donaldson J. The pathophysiology of trace metal neurotransmitter interaction in the CNS. *Trends in Pharmacol Sci* 1981; 1:75-77

Donaldson J, Cloutier T, Minnich JL and Barbeau A. Trace metals and biogenic amines in rat brain. *Advances in Neurology* 1974; 5:245-252

Donaldson J, Minnich JL and Barbeau A. Ouabain-induced seizures in rats: regional and subcellular localization of ^{3}H-ouabain associated with NA$^+$-K$^+$-ATPase in brain. *Can J Biochem* 1972; 50:888-896

Donaldson J, Minnich JL and Barbeau A. Effet du lithium et du rubidium sur les monoamines cérébrales de la souris. *Union Méd Can* 1973; 102:907-911

Donaldson J, Minnich JL, Izumi K and Barbeau A. Regional sodium-pump activity and epilepsy. *The Lancet* 1972; 1:1129-1130

Donaldson J, Labella FS and Gesser D. Enhanced autoxidation of dopamine as a possible basis of manganese neurotoxicity. *Neurotoxicology* 1981; 2:53-64

Donaldson J, St-Pierre T, Minnich J and Barbeau A. Seizures in rats associated with divalent cation inhibition of Na$^+$-K$^+$-ATPase. *Can J Biochem* 1971; 49:1217-1224

Donaldson J, St-Pierre T, Minnich J and Barbeau A. Determination of Na^+, K^+, Mg^{2+}, Co^{2+}, Zn^{2+} and Mn^{2+} in rat brain regions. *Can J Biochem* 1973; 51:87-92

Edsall DL, Wilbur FP and Drinker CK. The occurrence, course and prevention of chronic manganese poisoning. *J Industrial Hygiene* 1937; 1:183-193

Ehringer H and Hornykiewicz O. Verteilung von noradrenalin und dopamin (3-hydroxytyramin) im Gehirn des Menschen und ihr Verhalten bei Erkrankung des extrapyramidalen system. *Klin Wochschr* 1960; 38:1236-1238

Fehling C. Treatment of Parkinson's disease with L-DOPA: double blind study. *Acta Neurol Scandinav* 1966; 42:367-372

Flintzer H. Ueber gewerbliche manganvergiftung. *Arch f Psychiat* 1931; 93:84-115

Foley JM and Baxter D. On the nature of pigment grannules in the cells of the locus coeruleus and substantia nigra. *J Neuropath Exp Neurol* 1958; 17:586-598

Foradori A and Dinamarca M. Transferrina o transmanganina?: Definition de la proteina de transporte de manganeso en el plasma humano. *Revista Medicina de Chile* 1972; 100:148-153

Garcia-Carmona F, Garcia-Canovas F, Iborra JL and Lozano JA. Kinetic study of the pathway of melanization between L-DOPA and dopachrome. *Biochim Biophys Acta* 1982; 717:124-131

Graham, DG. Oxidative pathways for catecholamines in the genesis of neuromelanin and cytotoxic quinones. *Mol Pharmacol* 1978; 14: 633-643

Graham DG, Tiffany SM, Bell WR and Gutknecht WF. Autoxidation versus covalent binding of quinones as the mechanism of toxicity of dopamine, 6-hydroxydopamine and related compounds towards C 1300 neuro-blastoma cells *in vitro*. *Mol Pharmacol* 1978; 14:644-653

Greenhouse AH. Manganese intoxication in the United States. *Trans Am Neurol Assoc* 1971; 96:248-249

Grünstein AM and Popowa N. Experimentelle manganvergiftung. *Arch Psychiatr* 1929; 87:742-755

Gupta SK, Murthy RC and Chandra SV. Neuromelanin in manganese-exposed primates. *Toxicology Letters* 1980; 6:17-20

Hughes ER and Cotzias GC. Adrenocorticosteroid hormones and manganese metabolism. *Amer J Physiol* 1961; 201:1061-1064

Hughes ER, Cotzias GC and Cronkite EP. Total-body irradiation and manganese metabolism. *Nature* 1962; 193:792-793

Hughes ER, Miller ST and Cotzias GC. Tissue concentrations of manganese and adrenal function. *Amer J Physiol* 1966; 211:207-210

Hui KS, Davis BA and Boulton AA. Mass spectrometric identification of Cu, Zn, Fe, Co, Mn, Mg and Pb in mammalian brain. *J Neurosci Res* 1979; 4:169-175

Inoue N, Tsukada Y and Barbeau A. Behavioral effects in rats following intrastriatal microinjection of manganese. *Brain Res* 1975; 95:103-124

Izumi K, Donaldson J and Barbeau A. Yawning and stretching in rats induced by intraventricularly administered zinc. *Life Sci* 1973; 12:203-210

Izumi K, Donaldson J, Minnich JL and Barbeau A. Ouabain-induced seizures in rats: relationship to brain mono-amines. *Can J Biochem* 1973a; 51:198-203

Izumi K, Donaldson J, Minnich JL and Barbeau A. Ouabain-induced seizures in rats: modification by melatonin and melanocyte-stimulating hormone. *Can J Physiol Pharmacol* 1973b; 51:572-578

Izumi K, Donaldson J, Minnich JL and Barbeau A. Ouabain-induced seizures in rats: suppressive effects of taurine and γ-aminobutyric acid. *Can J Physiol Pharmacol* 1973c; 51:885-889

Johnson W, Schwartz G and Barbeau A. Studies on dystonia musculorum deformans. *Arch Neurol* 1962; 7:301-313

Keele BB, McCord, JM and Fridovich I. Superoxide dismutase from escherichia coli B - a new manganese-containing enzyme. *J Biol Chem* 1970; 245:6176-6181

Kenten RH and Mann PGJ. The oxidation of manganese by peroxidase systems. *Biochem J* 1950; 46:67-73

Kimura M, Yagi N and Itokawa Y. Effect of subacute manganese feeding on serotonin metabolism in the rat. *J Environ Path Toxicol* 1978; 2: 455-461

Kobert R. *Lehrbuch der Intoxikationen*. Berlin 1906

Kono Y, Takahashi M and Asada K. Oxidation of manganous pyrophosphate by superoxide radicals and illuminated spinach chloroplasts. *Arch Biochem Biophys* 1976; 174:454-461

Krivoy WA and Guillemin R. On a possible role of β-melanocyte stimulating hormone (β-MSH) in the central nervous system of the mammalia: an effect of β-MSH in the spinal cord of the cat. *Endocrinology* 1961; 69:170-175

Lai JCK, Leung TKC, Guest JF, Davison AN and Lim L. The effects of chronic manganese chloride treatment expressed as age-dependent, transient changes in rat brain synaptosomal uptake of amines. *J Neurochem* 1982; 38:844-847

Lai JCK, Leung TKC and Lim L. Brain regional distribution of glutamic acid decarboxylase, choline acetyltransferase and acetylcholinesterase in the rat: effects of chronic manganese chloride administration after two years. *J Neurochem* 1981; 36:1443-1448

Lai JCK, Leung TKC and Lim L. Activities of the mitochondrial NAD-linked isocitric dehydrogenase in different regions of the rat brain. *Gerontology* 1982; 28:81-85

Lal JCK, Minski MJ, Chan AWK, Lim L and Davison AA. Brain regional manganese distribution after chronic manganese treatment. *Biochem Soc Trans* 1981; p 228

Laker M. On determining trace element levels in man: the uses of blood and hair. *The Lancet* 1982; 2:260-262

Larsen NA, Pakkenberg H, Damsgaard E and Heydorn K. Topographical distribution of arsenic, manganese, and selenium in the normal human brain. *J Neuro Sci* 1979; 42:407-416

Larsen NA, Pakkenberg H, Damsgaard E, Heydorn K and Wold S. Distribution of arsenic, manganese, and selenium in the human brain in chronic renal insufficiency, Parkinson's disease, and amyotrophic lateral sclerosis. *J Neurol Sci* 1981; 51:437-446

Leach RM and Lilburn MS. Manganese metabolism and its function. *World Rev Nutr Diet* 1978; 32:123-134

Lerner AB, Shizume K and Bunding I. Mechanism of endocrine control of melanin pigmentation. *J Clin Endocrinol Metab* 1954; 14:1463-1509

Leung TKC, Lal JCK and Lim L. The regional distribution of monoamine oxidase activities towards different substrates: effects in rat brain of chronic administration of manganese chloride and of aging. *J Neurochem* 1981; 36:2037-2043

Lewy FH and Tiefenbach L. Die experimentalle manganperoxyde encephalitis and ihre sekundare autoinfektion. *Z Gesamte Neurol Psychiatr* 1921; 71:303-320

Martinez H and Bonilla E. Water intake and brain choline-acetyltransferase and acetylcholinesterase activities in manganese treated rats. *Neurobehav Toxicol Teratol* 1981; 3:277-280

Maynard LS and Cotzias GC. Partition of manganese among organs and intracellular organelles of the rat. *J Bioch Chem* 1955; 214:489-495

McGeer PL and Zeldowics LR. Administration of dihydroxyphenylalanine to Parkinsonian patients. *Canad Med Ass J* 1964; 90:463-466

Melri U, and Rahamimoff R. Neuromuscular transmission: Inhibition by manganese ions. *Science* 1974; 176: 308-309

Mella H. The experimental production of basal ganglia symptomatology in macacus rheaus. *Arch. Neurol. Psychiat.* 1924; 11:405-417

Mena I, Court J, Fuenzalida S, Papavasiliou PS, and Cotzias GC. Modification of chronic manganese poisoning — treatment with L-DOPA or 5-OH tryptophane. *New Engl. J. Med.* 1970; 282:5-10

Mena I, Marin O, Fuenzalida S and Cotzias GC. Chronic manganese poisoning — clinical picture and manganese turnover. *Neurology* (Minn) 1967; 17:128-136

Montplaisir J and Barbeau A. Serum tyrosine in Parkinson's disease. In: *Progress in Neurogenetics* Barbeau A and Brunette JR, eds. Excerpta Medica Foundation, Amsterdam 1969; 331-335

Murphy G and Abbona IM. Intoxication cronica por manganeso. *Prens. Med. Argent.* 1959; 46:845-850

Mustafa SJ and Chandra SV Levels of 5-hydroxytryptamine, dopamine and norepinephrine in whole brain of rabbits in chronic manganese toxicity. *J. Neurochem.* 1971; 18:931-933

Narabayshi H. Personal communication, 1981.

Neff NH, Barrett RE and Costa E. Selective depletion of caudate nucleus dopamine and serotonin during chronic manganese dioxide administration to squirel monkeys. *Experientia* 1969; 25:1140-1141

Papavasiliou PS and Cotzias GC. Neutron activation analysis: the determination of manganese *J Biol Chem* 1961; 236:2365-2369

Papavasiliou PS, Miller ST and Cotzias GC. Role of liver in regulating distribution and excretion of manganese. *Amer J Physiol* 1966; 211 211-216

Papavasiliou PS, Miller ST and Cotzias GC. Functional interactions between biogenic amines. 3', 5'-cyclic AMP and manganese *Nature* 1968; 220:74-75

Papavasiliou PS, Miller ST, Cotzias GC, Kraus HW, Hsieh RS. Sequential analysis: manganese, catecholamines and L-DOPA induced dyskinesia. *J Neurochem* 1975; 25:215-219

Parnitzke KH and Peiffer J. Zur klinik und pathologischen anatomia chronischer braunstein vergiftung. *Arch Psychiat U Nervenkr* 1959; 192:405-429

Peñalver R. Manganese poisoning — The 1957 Ramazzini Oration. *Ind Med Surg* 1955; 24:1-

Peñalver R. Diagnosis and treatment of manganese intoxication. *AMA Arch Industr Health* 1957; 16:64-66

Pentschew A. Implications of experimental manganese encephalopathy on neurology and neuropathology. *Rev. Neuro. Psiquiat.* 1963; 26:139-143

Pentschew A, Ebner FF and Kovatch RM. Experimental manganese encephalopathy in monkeys — a preliminary report. *J Neuropath Exp Neurol* 1963; 22:488-499

Prasad KN. Manganese inhibits adenylate cyclase activity and stimulates phosphodiesterase activity in neuroblastoma cells: its possible implication in manganese poisoning. *Exper Neur* 1974; 45:554-557

Prasad KN, Johnson HA and Cotzias GC A cytoplasmic organelle of melanocytes. *Nature* 1965; 205:525-526

Rodier J. Considérations sur le manganisme au Maroc. *Maroc Med.* 1950; 29:817-826

Rodier J. Manganese poisoning in moroccan miners. *Brit J Industr Med* 1955; 12:21-35

Roy M, Boyer L, and Barbeau A. A prospective study of 50 cases of familial Parkinson's disease. *Can J Neurol Sci* 1983; 10:37-42

Sano I, Gamo T, Kakimoto K, Taniguchi M, Takesada M and Nishinuma K. Distribution of catechol compounds in human brain. *Biochem Biophys Acta* 1959; 32:586-588

Saxena K. The acute effect of manganese chloride on the central nervous system of rats. *Ind J Industrial Med* 1967; 13:66-72

Scherer JH. Melanin pigmentation of the substantia nigra in primates. *J Comp Neurol* 1939; 71:91-98

Scheving LE, Pauly JE, Kanabrucki EL and Kaplan E. A 24-hour rhythm in serum copper and manganese levels of normal and adrenal medullectomized adult male rats. *Texas Reports on Biology and Medicine* 1968; 26:341-347

Scruton MC. Purification and some properties of a protein containing bound manganese (Avimanganin). *Biochem.* 1971; 10:3897-3905

Seelert H. Ein Fall chronischer manganvergiftung. *Monatschr P Psychr U Neurol* 1913; 24:82-92

Shukla GS and Chandra SV. Striatal dopamine turnover and L-DOPA treatment after short-term exposure of rats to manganese. *Arch. Toxicol.* 1981; 47:191-196

Stadler H. Zur Histopathologie des Gehirns bei Manganvergiftung. *Ztschr f d ges Neurol u Psychiat* 1936; 154:62-76

Suzuki M and Wacker WEC. Determination of manganese in biological materials by atomic absorption spectroscopy. *Analytical Biochem* 1974; 57:605-613

Tanaka S and Lieben J. Manganese poisoning and exposure in Pennsylvania. *Arch Environ Health* 1969; 19:674-684

Trendtel I. Zur frage des manganismus. *Mschr. Unfallheilk* 1936; 43:69-84

Tsukada Y, Inoue N, Donaldson J and Barbeau A. Suppressive effect of various amino acids against ouabain-induced seizures in rats. *Can J Neurol Sci* 1974; 1:214-221

Van Bogaert L and Dallemagne MJ. Approches expérimentales des troubles nerveux du manganisme. *Mschr Psychiat Neurol* 1943; 111:60-89

Vanella A, Geremia E, D'Urso G, Tiriolo P, Di Silvestro I, Grimaldi R and Pinturo R. Superoxide dismutase activities in aging rat brain. *Gerontology* 1982; 28:108-113

Van Woert MH. Spectral studies of the pigment of the human stustantia nigra. *Advances in Neurology* 1974; 5:306-310

Van Woert MH and Ambani LM. Biochemistry of Neuromelanin. *Advances in Neurology* 1974; 5: 215-223

Van Woert MH, Nicholson A and Cotzias GC. Mitochondrial functions of polymelanosomes. *Comp Biochem Physiol* 1967; 22:477-485.

von Jaksch, R. Uber mangantoxikosen und manganophobie. *Munch Med Wochenschr* 1907; 20:969-972

von Oettingen WF. Manganese: its distribution, pharmacology and health hazards. *Physiol Rev* 1935; 15:175-201

Voss H. Progressive bulbarparatyse und lateralsklerose nach chronischer manganvergiftung. *Arch Geerhepath Gewerbehyg* 1939; 9:464-476

Weiner WJ, Nausieda PA and Klawans HL. Effect of chlorpromazine on central nervous system concentrations of manganese, iron and copper. *Life Sci* 1977; 20:1181-1186

Weiner WJ, Nausieda PA and Klawans HL. The effect of levodopa, lergotrile, and bromocriptine on brain iron, manganese and copper. *Neurology* 1978; 28:734-737

Weisiger RA and Fridovich I. Superoxide dismutase organelle specificity. *J Biol Chem* 1973; 248: 3582-3592

Whitlock CM, Amuso SJ and Bittenbender JB. Chronic neurological disease in two manganese steel workers. *Am Ind Hygiene Ass J* 1966; 27: 454-459

Wynter J. The prevention of manganese poisoning. *Indust Medic Hygien* 1962; 31:308-310

third series of experiments. The aim of these studies was to discover whether manganese offered promise as a generator of free radicals in some other system of obvious biological significance. *Melanin* had been described as indeed being rich in free radicals. Hair, selected on the basis of its colour, was found to contain increasing concentrations of free radicals with increasing darkness of the specimens. Melanin was thus studied biochemically. It was shown (Cotzias, Papavasiliou and Miller, 1964) that dark structures of every individual examined contained much higher concentrations of manganese than did the white ones. In view of this finding *manganese was assigned to the final, auto-oxidative steps of melanogenesis* and a large series of investigations, particularly with Borg and Van Woert, led to the conclusion that melanosomes, or specific cytoplasmic organelles (Polymelanosomes), are to melanocytes what mitochondria are to non-pigmented cells: both contain large concentrations of manganese and free radicals (Prasad, Johnson and Cotzias, 1965; Van Woert, Nicholson and Cotzias, 1967).

In primates the greatest intensity of pigmentation is in the *substantia nigra* (Scherer, 1939). It is well known, of course, that severe depigmentation occurs in the substantia nigra, in the locus coeruleus and in the dorsal nucleus of the vagus in Parkinson's disease, but it is less well recognized that these areas are intact in albinos (Foley and Baxter, 1958). The cells of the substantia nigra appear to be melanocytes and to contain free radicals (Borg, 1974). Spectral studies of the pigment of the substantia nigra led to a better understanding of the biochemistry of neuromelamin (Van Woert, 1974; Van Woert and Ambani, 1974). Thus no tyrosinase is present in the substantia nigra. On the other hand, dopamine is easily auto-oxidized to form melanin. The semiquinone free radical of melanin can act as an electron acceptor. Finally, it was shown that melanin is also reactive with several drugs, particularly phenothiazine compounds. The *link between manganese, melanin, and the extrapyramidal system* had now been made, (Cotzias, 1966) and it was permissible to study the metabolism of this trace element in a variety of disorders of movement. This would be the next, and most important, phase of Dr. Cotzias's work.

The study of miners exposed to *chronic manganese overload* was the natural follow-up to these basic observations, particularly since many of the miners presented with extrapyramidal symptoms. This was accomplished with the cooperation of Chilean neurologists (Cotzias, Borg and Bertinchamps, 1960; Mena *et al*, 1967; Cotzias *et al*, 1968). It was shown that chronic manganese poisoning differs from Wilson's disease. Surprisingly tissue burdens of manganese were found to be higher in "healthy" miners than in ex-miners removed from the mines because of their extrapyramidal disease or mental changes ("locura manganica"). These symptoms included bradykinesia, postural difficulties, prominent rigidity, some tremor and occasionally significant dystonia. Turnover of radioactive manganese was significantly faster in "healthy" exposed miners than in the extrapyramidal patients or than in control normal subjects. Hair manganese concentration was only elevated in the "healthy" exposed miners. Thus these observations demonstrated that manganese overload was present only during exposure and was not in parallel to the presence of extrapyramidal symptoms. *It is thus not necessary to maintain high tissue levels of manganese to have neurological disease.* This capital, and unexpected, finding differentiated chronic manganese poisoning from Wilson's disease and, as now predicted, chelating agents were of no value in treatment. It was thus necessary to turn to other possible therapeutic approaches.

Melanocyte-stimulating hormone (β-MSH) is capable, in integumental melanocytes, of increasing melanin deposition (Lerner, Shizume and Bunding, 1954). This phenomenon is well-known in frogs. β-MSH can also reverse the effects of chlorpromazine (Krivoy and Guillemin, 1961). For these two reasons, β-MSH was tried in Parkinsonian patients in an effort to repigment the damaged substantia nigra (Cotzias, Van Woert and Schiffer, 1967). Unfortunately the Parkinsonian state was reversibly aggravated by the administration of this hormone and this approach was abandoned.

The L-Dopa Story

At this stage in our story it would be of interest to abandon Cotzias and his studies and to turn back a few years to my own laboratory to understand the background for the next important phase of the Cotzias saga. As I was completing my post-graduate studies in Montreal in 1956 and 1957, important developments were occuring in the understanding of biochemical events within the basal ganglia of the brain. Carlsson *et al.* (1958), Bertler and Rosengren (1959), as well as Sano and collaborators (1959) had demonstrated the presence of dopamine within the brain, and particularly within the basal ganglia. While training in neurology in Chicago in 1958, I had the pleasure of listening to Professor Carlsson lecturing at a meeting in Washington. At the time, I had been studying catecholamine excretion in various neurological disorders, and had found abnormalities in the urine of Parkinsonian patients (Barbeau, 1961). After that lecture, I decided to study the *specific* excretion of *dopamine* in Parkinson's disease. In these preliminary studies, as well as in more detailed later investigations in Professor Sourkes' laboratory in 1959 and 1960 (Barbeau, Murphy and Sourkes, 1961) we were able to clearly demonstrate a significantly decreased urinary excretion of dopamine in Parkinson's disease. It is of interest to note that while I was carrying out these experiments, one of my good friends, also training at the University of Chicago, was Dr. Melvin Van Woert, who was to play such an important role in the study of melanin in Dr. Cotzias' laboratory and who, throughout the years between 1959 and 1967, kept in touch with me and my DOPA studies.

At the same time and independently, Ehringer and Hornykiewicz (1960) reported similar dopamine deficits in the brain of Parkinsonian patients, particularly in the basal ganglia. It was thus natural for both our groups to attempt replacement therapy with dopamine, but this failed because of the blood-brain barrier to dopamine itself. We then turned to the natural precursor: L-DOPA. (Barbeau, 1961; Birkmayer and Hornykiewicz, 1961; Barbeau 1962), either intravenously or orally; following the crucial observations of Carlsson, Lindquist and Magnusson (1957) who had demonstrated that L-DOPA could reverse the extrapyramidal symptoms induced by reserpine. As is well-known, these early experiments in Parkinson's disease were successful and the physiological reversal of rigidity and akinesia in Parkinson's disease was clearly demonstrated. Although two similar studies were subsequently negative (McGeer and Zeldowicz, 1964; Fehling 1966), by 1968 the bulk of 32 publications were supportive of these findings (Barbeau, 1969). I had the privilege to present our own results with L-DOPA in Brookhaven, in Dr. Cotzias' department, on two occasions, in 1963 and 1966, and to discuss with him the therapeutic potential of this approach, which was then extremely expensive. By the second visit to Long Island, Dr. Cotzias had just completed his β-MSH studies and was postulating that the hormone was shifting